REMARKS

Favorable reconsideration is respectfully requested.

The claims are 1 and 2.

Claims 1 and 2 have been rejected under 35 U.S.C. 102(b) as unpatentable over Guha et al.

This rejection is respectfully traversed.

While the rejection appears to have relied on an abstract of the Guha et al report, Applicants have studied the original full text of the report (copy attached) and comment as follows.

The rejection contends that the reference teaches a method of culturing edible fungi and that the claims are identical to the abstract of the Guha report. Applicants wish to point out several important and unobvious differences between the method of claims 1-2 and the disclosure of Guha et al.

The most important difference is in respect of the manner of ensuring an aerobic condition of the culturing system. In the inventive method, the aerobic culturing condition is maintained by blowing or bubbling sterilized air of a specified oxygen concentration into the culture medium.

In Guha's method, on the other hand, no such a measure is undertaken so that the culturing system is only incompletely aerobic, if not anaerobic. In Guha, namely, the liquid culture medium is taken in an Erlenmeyer flask and the flasks after inoculation (presumably stopped with a sterilized cotton plug) are shaken on a rotary shaker (page 82, right column, lines 2-7) to ensure only limited contact of the atmospheric air with the surface of the liquid culture medium.

Other differences can be found in the nutrient constitution of the culture medium. In the inventive method, the carbon source is essentially a combination of sucrose and maltose each in a specified concentration according to claim 1. In Guha, on the other hand, no such a combination of these two disaccharides is taught or suggested, even though various saccharides including sucrose and maltose were tested (page 82, left column, top paragraph) because the object of the

Guha's experiments was to study the effect of different carbon sources by individually testing the different saccharides (Table 1).

Another difference, though minor, in the nutrient constitution in the culture medium, is found in the amount of yeast extract which is always 2 g/liter in Guha (page 82, left column, line 3 from the bottom) while the amount is 0.3-1.2 g/liter (claim 1).

The foregoing reasons, it is apparent the rejection on Guha is unattainable and should be withdrawn.

No further issues remaining, allowance of this application is respectfully requested.

If the examiner has comments or proposals for expediting prosecution, please contact undersigned at the telephone below.

Respectfully submitted,

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Effect of Different Carbon Compounds on the Submerged Production of *Agaricus campestris* Mycelium

Humfeld and Sugihara[1] found that *A. campestris* could utilise different carbon sources when grown in the submerged culture. Good yields were obtained from D-glucose, D-galactose, D-mannose, D-fructose, maltose, D-xylose, L-arabinose, dextrin, mannitol, sucrose and soluble starch. Szuecs[2] patented a process for the production of mycelium and essence of *Psalliota campestris* in which he claimed that corn syrup, hydrolysed starch, cream of wheat, maltose, sucrose and related compounds serve as good carbon sources in complex media. Litchfield *et al*[3]. showed that *Morchella hortensis*, *M. crassipes* and *M. esculenta* grow well in $(NH_4)_2HPO_4$ — corn steep liquor basal medium containing glucose or maltose as carbon source. The first two organisms also grew readily in lactose containing medium while *M. esculenta* did not. They showed that yield of mycelial materials depended on C:N ratio in the medium. Ferry *et al.*[4] studied the carbon nutrition of some *Mycorhizal boletus* species in synthetic medium and showed that glucose is the best supporter of growth. In all the studies referred to above only the growth of the organism was taken into consideration, the relative effect of different carbon compounds on the protein content of the mycelia was not measured. The present investigation was undertaken to determine the effects of different carbon sources on the growth of *Agaricus campestris* in a synthetic media. The relative efficiency of these compounds on yields of the mycelia were also compared. The results indicate some interesting differences from the previous reports with different strains of *A. campestris*. This suggests the variable nutritional habits of this fungus with variation of strain as indicated in our previous paper[5].

A strain of *A. campestris* developed in this laboratory by tissue culture technique was used throughout the experiment. The culture maintenance and inoculum development for fermentation experiments has been described earlier[5]. To test the utilization of different C-sources, fermentations were carried out in a medium containing C-source, 20 g; $NaNO_3$, 10 g; KCl, 0.5 g; $MgSO_4$, $7H_2O$, 0.5 g; KH_2PO_4, 1 g; Fe SO_4, $7H_2O$—trace, yeast extract, 2 g (as vitamin source), per litre of distilled water. The pre-sterilisation pH of the medium was adjusted to 6.0. The medium (50 ml) was dispensed in 250 ml Erlenmeyer flask and heat sterilized. The flask containing no C-source was taken as control. The flasks were inoculated with approximately equal amounts of mycelia and incubated on a rotary shaker (120 cycles/min) at room temperature (30°C), growth being permitted for 7 days.

The growth of the organism was measured gravimetrically as described before. The protein content of dried mycelium was measured by determining N by microkjeldahl procedure[6]. Since nucleic acid N would interfere in this assay procedure seriously, giving high values for protein in the crude cellular material, the dried mycelia was suspended in 5 per cent trichloroacetic acid and held at 90-95°C for 20 min. This treatment hyolrolysed the nucleic acids without affecting the protein[7]. The mixture was cooled and centrifuged. The protein was recovered in the sediment, which was washed with TCA twice. The nitrogen content of the protein fraction was determined. The protein content was calculated by assuming that mushroom protein contained 16 per cent nitrogen.

Glucose, fructose, xylose, mannitol and glycerol content of the medium before and after fermentation were measured iodometrically after periodate reaction[8]. Clear supernatant free of mycelial material was taken for periodate oxidation.

The effect of C-sources on the growth of *A. campestris* mycelia are summarized in Table 1. It shows that mannitol is the best supporter of growth. Glucose is also almost equally efficient in this respect. Economic coefficient which is an index of utilisation of carbon compounds for the building up of cellular material is high for xylose and mannitol (Table 2). These values of glucose and fructose are comparable and are little lower than those from xylose and mannitol. From the extent of utilisation, efficiency of recovery of carbon in cell material, and protein content of mycelia produced; mannitol appears to be the best carbon source for this strain. Out of 3 different disaccharides tested maltose could support the growth to the maximum extent, lactose and sucrose are utilised at a slow rate. Probably the hydrolysis of these disaccharides is the rate limiting step in their

TABLE 1. UTILISATION OF DIFFERENT C-SOURCES BY A. CAMPESTRIS

Carbon source	Dry wt. of mycelium in gms/lit.	% of protein in mycelium	Protein yield/L medium in mg.	Final pH of the broth
Glycerol	1.8	35.4	837.2	4.2
L (+) Arabinose	1.76	31.7	557.2	5.0
D (+) Xylose	3.18	27.19	864.6	5.4
D-Glucose	3.44	28.2	970	5.0
D-Galactose	0.46	Not estimated	...	5.6
D (+) Fructose	2.6	28	728	6.0
L (+) Rhamnose	0.12	Not estimated	...	5.8
Mannitol	3.80	31	1178	5.4
Maltose	2.4	27.6	662.4	5.4
Sucrose	1.4	24	336	6.0
Lactose	1.94	27	523.8	4.4
Raffinose, Pentahydrate	0.10	Not estimated	...	5.8
Sodium acetate trihydrate	0.16	,,	...	6.0
Sodium citrate	0.14	,,	...	6.0
Control (without C-source)	0.10	,,	...	5.8

utilisation. Moreover galactose utilisation is very poor which may further explain the low growth in lactose containing medium. The carbon sources of the growth medium has some influence on the protein content of cellular mycelia but this influence is not well marked as that of nitrogen sources.

TABLE 2. EFFICIENCY OF SUGAR UTILISATION BY A. CAMPESTRIS

Substrate	% of supplied carbohydrate utilised during fermentation	Economic coefficient g of mycelium produced per g of sugar consumed × 100
Glucose	94.5	23.9
Fructose	89.2	20.8
Xylose	71.5	30.7
Glycerol	50.0	24.2
Mannitol	66.2	29.0

Like the strain tested by Humfeld and Sugihara[1] the present strain was unable to utilize L-rhamnose. Also, it could not utilise raffinose and two organic acids tested.

It is interesting to note that the present strain could not utilise galactose, whereas lactose is utilised though not very efficiently. These are in contrast to the observation of Humfeld and Sugihara[1] who observed that for their strain galactose has good carbon source and lactose is unutilisable. These findings support our previous suggestion that nutritional pattern of cultured *A. campestris* varies in different strains[5].

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22 December 1970

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Serial No.: 10/054,905 **Filing Date:** January 25, 2002
Title: METHOD FOR CULTURING EDIBLE FUNGUS

Receipt of the following papers is acknowledged:

1. Response
2. Copy of Report (Reference)

Date: December 31, 2003 **Attorney:** MJ/tg **[Check No._____]**

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